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ANTIHELMINTIČKI POTENCIJAL ETARSKOG ULJA KORIJANDERA (*CORIANDRUM SATIVUM L.*) KOD OVACA

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Sažetak

Razvoj antihelmintičke rezistencije kod gastrointestinalnih nematoda ovaca na komercijalne antihelmintike je doveo do ogromnih ekonomskih gubitaka u mnogim delovima sveta. Zbog toga se aktivno traga za novim održivim strategijama za kontrolu ovih parazita, koje uključuju i primenu različitih alternativa poput biljnih etarskih ulja. Cilj ovog istraživanja je bio da se odredi hemijski sastav etarskog ulja korijandera (*Coriandrum sativum L.*) i da se ispita njegov antihelmintički potencijal protiv gastrointestinalnih nematoda ovaca. Kvalitativna i semikvantitativna hemijska karakterizacija etarskog ulja izvršena je primenom gasne hromatografije kuplovane sa masenospektrometrijskom detekcijom (GC-MS analize). Antihelmintički potencijal etarskog ulja je ispitan pomoću *in vitro* testa izleganja larvi (EHT), koji je sproveden sa osam različitih koncentracija ispitanog ulja (50; 12,5; 3,125; 0,781; 0,195; 0,049; 0,025 i 0,0125 mg/mL). Pri tome je za pozitivnu kontrolu korišćen tiabendazol u koncentracijama 0,025 i 0,0125 mg/mL, a za negativnu emulgator 3% Tween 80 v/v i destilovana voda. GC-MS analizama je otkriven bogat hemijski sastav ispitanog ulja, pri čemu je pronađeno ukupno 17 različitih sastojaka iz različitih hemijskih grupa, od čega je 15 identifikovano. Dominantan sastojak je bio linalool (84,5%) uz γ -terpinen

(5,04%), kamfor (3,26%) i α -pinen (2,88%). Rezultati EHT su pokazali visok antihelmintički potencijal ulja korijandera sa inhibicijom izleganja larvi odnosno ovoidnim efektom od 29,0-88,7% u zavisnosti od koncentracije, uz dobijenu IC_{50} koncentraciju od 0,04 mg/mL i jasan dozno-zavisni efekat ($R^2=0,90$). Ova studija je potvrdila moguću značaj botaničkih antihelmintika u održivoj kontroli nematoda kod životinja i borbi protiv antihelmintičke rezistencije. Dobijeni rezultati ukazuju na moguću ulogu korijandera u budućim terapijskim pristupima infekcijama izazvanih gastrointestinalnim nematoda ovaca, nakon daljih *in vivo* ispitivanja i studija toksičnosti.

Ključne reči: gastrointestinalne nematode, antihelmintička rezistencija, etarsko ulje, korijander, gasna hromatografija, test izleganja larvi.

UVOD

Gastrointestinalne nematode na različite načine dovode do negativnih efekata kod ovaca, od subkliničkih gubitaka na težini i opadanja proizvodnje mleka, mesa i vune, pa sve do pojave anemije, dijareje, anoreksije, gubitaka proteina, smanjenog fertiliteta, a u težim slučajevima dolazi i do uginuća (Giovanelli i sar., 2018; Bosco i sar., 2020; Belecké i sar., 2021). Kontrola infekcija izazvanih ovim parazitima se i dalje uglavnom gotovo isključivo oslanja na upotrebu komercijalnih antihelmintičkih lekova poput benzimidazola, makrocikličnih laktona i imidazotiazola (Dyary, 2018). Iako su ovi lekovi decenijama uspešno korišćeni u terapiji pomenutih infekcija (Kaplan, 2020), njihova neracionalna upotreba u smislu učestalih i intenzivnih tretmana, visokih ili niskih doza ili uzastopnog korišćenja antihelmintika iz samo jedne klase dovela je do razvoja rezistencije kod gastrointestinalnih nematoda (Bosco i sar., 2018; Pinto i sar., 2019; Belecké i sar., 2021). Razvoj rezistencije je doveo do opadanja efikasnosti pomenutih lekova i posledičnih velikih ekonomskih gubitaka usled smanjene proizvodnje kod životinja i uginuća, ali i troškova lečenja (Szewc i sar., 2021). Zbog svega navedenog, gastrointestinalne nematode predstavljaju jednu od najvećih prepreka sa kojima se suočava moderno ovčarstvo (Giovanelli i sar., 2018).

Sve veći i izraženiji problem rezistencije zahteva osmišljavanje novih strategija za kontrolu pomenutih parazita, a koje se danas uglavnom baziraju na integrisanom pristupu terapiji (Keeton, 2016). Ovaj pristup podrazumeva sa jedne strane racionalnu upotrebu antihelmintika odnosno ciljane tretmane (tretman samo određenih stada) i ciljane selektivne tretmane (tretman samo pojedinih životinja koje zahtevaju lečenje unutar stada), uz kombinaciju više antihelmintika ili njihovu rotaciju (Calvete i sar., 2020). Sa druge strane i uz racionalno korišćenje

komercijalnih preparata, integrisan pristup podrazumeva i uvođenje različitih alternativa poput selekcije genetski rezistentnih životinja, upravljanje pašnjacima, nutritivne manipulacije, biološku kontrolu (upotrebu nematofagnih gljiva ili bakterija), razvoj vakcina kao i upotrebu botaničkih antihelmintika (Zeineldin i sar., 2018; Pinto i sar., 2019). Pri tome, od navedenih alternativa se fitoterapija se s obzirom na pokazane rezultate smatra posebno obećavajućom (Borges i Borges, 2016). Ona podrazumeva upotrebu celih biljaka ili njihovih delova u hrani za životinje, ali i različitih biljnih produkata poput ekstrahata i etarskih ulja.

Etarska ulja predstavljaju aromatične, koncentrovane i složene mešavine isparljivih i nepolarnih jedinjenja izolovanih iz biljnog materijala (Štrbac i sar., 2022a). Prisutna su u specijalizovanim ćelijama ili žlezdama određenih biljaka i imaju ulogu u njihovoj zaštiti od predatora i štetočina, kao i u privlačenju oprašivača (Butnariu i Sarac, 2018). U veterinarskoj medicini, etarska ulja se sve češće koriste u tretmanu i prevenciji različitih oboljenja, iako prevashodno kod monogastričnih životinja poput svinja i živine (Mucha i Witkovska, 2021), ali i kod pasa i mačaka (Štrbac i sar., 2021). Međutim, sve je više dokaza i o antihelmintičkom efektu etarskih ulja protiv gastrointestinalnih nematoda ovaca (Štrbac i sar., 2022b). Njihov biološki efekat potiče od bogatog hemijskog sastava i jedinjenja koja pripadaju različitim grupama poput terpena, terpenoida ili fenilpropanoidnih jedinjenja (Dhifi i sar., 2016, Fokou i sar., 2020).

Korijander (*Coriandrum sativum* L.) predstavlja aromatičnu, zeljastu jednogodišnju biljku iz porodice *Apiaceae* (Chahal i sar., 2017). Ima dugu istoriju korišćenja u kulinarstvu kao izvor aromatičnih jedinjenja sa antibakterijskim, antigljivičnim i antioksidativnim delovanjem, pa je koristan u pripremi hrane kao aroma i pomoćno sredstvo (Mandal i Mandal, 2015). Poznat je i po širokom spektru lekovitih svojstava i koristi se kod različitih gastrointestinalnih tegoba poput anoreksije, dispepsije, nadimanja, dijareje i povraćanja. Plod korijandera je takođe poznat kao rashladno sredstvo, tonik, diuretik i afrodizijak, dok se njegovo etarsko ulje smatra korisnim kod nadimanja, reumatizma i neuralgije, uz poznata antimikrobna, antioksidativna i pesticidna svojstva (Chahal i sar., 2017).

Cilj ovog istraživanja je bio ispitati antihelmintičku aktivnost etarskog ulja korijandera protiv gastrointestinalnih nematoda ovaca uz određivanje njegovog hemijskog sastava i identifikaciju jedinjenja značajnih za antihelmintičku aktivnost.

MATERIJALI I METODE

Analiza hemijskog sastava

Etarsko ulje korijandera je nabavljeno iz Instituta za ratarstvo i povrtarstvo Novi Sad. Kvalitativna i semikvantitativna hemijska karakterizacija etarskog ulja izvršena je na Prirodno-matematičkom fakultetu u Novom Sadu primenom gasne hromatografije kuplovane sa masenospektrometrijskom detekcijom (GC-MS). U gasni hromatograf (Agilent Technologies series 6890) injektovan je 1 μ L rastvora ispitivanog ulja (10 μ L/mL u heksanu) u split modu, uz split odnos 1:10, pri temperaturi od 250°C. Komponente su razdvojene na nepolarnoj poli (tetrametil-1,4-silfenilensiloksanskoj) koloni HP-5 ms (Agilent Technologies) dimenzija 30m \times 0,25mm, debljina sloja 0,25 μ m. Kolona je eluirana u temperaturno-programiranom režimu, uz startnu temperaturu od 50°C, rampu 8°C/min do 120°C, 15°C/min do 230°C, 20°C/min do 270°C/min, i zadržavanje na finalnoj temperaturi od 16,92 min (ukupno vreme analize 35 min). Kao gas nosač korišćen je helijum visoke čistoće (5.0) u režimu konstantnog protoka od 1,0 mL/min. Efluent je preko transfer-linije održavane na 280°C prosleđen u maseni spektrometar sa elektronskom jonizacijom (Agilent Technologies series 5975). Parametri masenog spektrometra bili su: energija elektrona 70eV, temperatura jonskog izvora 230°C, temperatura kvadrupola 150°C. Primenjen je scan mod akvizicije, u m/z opsegu 35-400, i uz solvent delay od 2,30 min. U cilju postizanja boljeg slaganja između eksperimentalnih i bibliotečkih spektara, korišćen je standard spectra tune. Podaci su obrađeni pomoću Agilent Technologies MSD ChemStation softvera (revizija E01.01.335) u kombinaciji sa AMDIS (ver. 2.64) i NIST MS Search softverom (ver. 2.0d). AMDIS je korišćen za dekonvoluciju masenih spektara koeluirajućih jedinjenja, a NIST MS Search je obezbedio algoritam za bibliotečku pretragu komplementaran PBM algoritmu ChemStation-a. Za identifikaciju masenih spektara korišćene su spektralne biblioteke Wiley Registry of Mass Spectral Data 7 th Edition i NIST/EPA/NIH Mass Spectral Library 05. Identitet jedinjenja potvrđen je poređenjem linearnih retencionih indeksa sa literaturnim podacima. Relativni udeli jedinjenja određeni su metodom normalizacije, na osnovu površine pikova u TIC.

Test izleganja larvi

Antihelmintički potencijal etarskog ulja korijandera protiv gastrointestinalnih nematoda ovaca je ispitan uz pomoć testa izleganja larvi (eng. *egg hatch test*, EHT), koji meri uticaj primene neke supstance na inhibiciju izleganja larvi iz jaja parazita, odnosno ovoidni efekat. Istraživanje je sprovedeno u regionalnom centru za praćenje parazitoza KREMOPAR (Eboli, Salerno, južna Italija). Za izolaciju

jaja nematoda su korišćeni uzorci fecesa ovaca uzimani direktno iz rektalne ampule, od ovaca sa dve odvojene farme u ovom regionu, a jaja nematoda su izolovana uz pomoć tako zvane metode oporavka (eng. *recovery method*) (Bosco i sar., 2018).

Uzorci fecesa su najpre homogenizovani i filtrirani pod tekućom vodom kroz sita sa otvorima različitih veličina (1 mm, 250 μ m, 212 μ m i 38 μ m), u cilju odvajanja jaja iz fecesa. Jaja zadržana na situ najmanje veličine su potom isprana sa destilovanom vodom u epruvete i centrifugovana na 1500 RPM, nakon čega je talog otklonjen. Nakon toga je izvršeno centrifugiranje kako bi jaja koja su isplivala na površinu, bila izolovana u nove epruvete u koje je dodata destilovana voda. Na kraju, dobijeni rastvori su centrifugovani još nekoliko puta kako bi se otklonio debris i dobio vodeni rastvor sa jajima.

Test izleganja larvi je sproveden prema uputstvima iz literature (Ferreira i sar., 2018) uz određene modifikacije. Testirano je 8 različitih koncentracija (50; 12,5; 3,125; 0,781; 0,195; 0,049; 0,025 i 0,0125 mg/mL) ulja korijandera, pri čemu su se koristile ploče sa jažicama. U jažicama su pravljene rastvori navedenih opadajućih koncentracija ulja emulgovanih u 3% Tween-uu 80, v/v, i potom dopunjavane sa dobijenim vodenim rastvorom sa jajima (oko 40 ml odnosno 100-150 jaja po jažici) i destilovanom vodom do ukupne količine od 0,5 mL po jažici. Potrebna količina ulja za najveću koncentraciju je određena računski, a potom su se metodom razblaživanja dobile ostale koncentracije.

Kao pozitivna kontrola je korišćen tiabendazol u koncentraciji 0,025 mg/mL i 0,0125 mg/mL, a kao negativne 3% Tween 80, v/v i destilovana voda. Sve koncentracije ulja i kontrole su ispitane u ukupno tri ponavljanja. Nakon pravljenja pomenutih rastvora, ploča sa jažicama je inkubirana na 27°C tokom 48h, a nakon toga su dodate 1-2 kapi Lugolovog rastvora u svaku jažicu, kako bi se zaustavilo izleganje larvi nematoda iz jaja. Napravljeni rastvori su potom posmatrani pod mikroskopom sa ciljem brojanja jaja i izleglih larvi pri svakoj koncentraciji ulja i kontrola.

Statistička obrada

Procenat inhibicije izleganja jaja u larveni oblik je računat pomoću sledeće formule (Coles i sar., 1992; Pinto i sar., 2019):

$$IH = \text{broj jaja} / (\text{broj jaja} + \text{broj larvi}) \times 100\%$$

Nakon toga je krajnja vrednost za svaku koncentraciju i kontrole računata kao aritmetička sredina između tri ponavljanja. Za međusobno poređenje dobijenih vrednosti između različitih koncentracija ispitanog ulja, kao i za njihovo poređenje sa kontrolama korišćena je jednofaktorijalna analiza varijanse sa post hoc Takijevim testom ($p < 0,05$). Sa druge strane, za određivanje koncentracije

koja inhibiše 50% jaja za razvoj u larveni oblik korišćena je nelinearna regresija i logaritamska distribucija (Ferreira i sar., 2018; Štrbac i sar., 2022a). Statističke analize su rađene u programu GraphPad Prism 8.4.3. (GraphPad Holdings, LLC, San Antonio, CA, USA).

REZULTATI

Analiza hemijskog sastava

Rezultati sprovedene hemijske analize sastava uz pomoć gasne hromatografije i masene spektrometrije su pokazale bogat hemijski sastav etarskog ulja korijandera. Ukupan broj jedinjenja je bio 17 od kojih je 15 identifikovano (Tabela 1), iz različitih hemijskih grupa, a dominantna jedinjenja su bila linalool (84,53%), γ -terpinen (5,08%), kamfor (3,26%) i α -pinen (2,88%).

Tabela 1 Hemijski sastav etarskog ulja korijandera određen uz pomoć gasne hromatografije i masene spektrometrije

Aritmetički retencijski indeks	Jedinjenje	% Ukupne površine vrha
925	α -Tujon	0,02
932	α -Pinen	2,88
946	Kamfen	0,30
971	Sabinen	0,02
976	β -Pinen	0,21
989	Mircen	0,37
1016	α -Terpinen	0,05
1023	p-Cimen	1,08
1027	Limonen	1,12
1029	1,8-Cineol	0,14
1056	γ -Terpinen	5,08
1087	Terpinolen	0,30
1100	Linalool	84,53
1143	Kamfor	3,26
1165	<i>Nepoznato</i>	0,12
1176	Terpinen-4-ol	0,27
1191	<i>Nepoznato</i>	0,16

Test izleganja larvi

Etarsko ulje korijandera je pokazalo visok antihelmintički potencijal protiv gastrointestinalnih nematoda ovaca sa inhibicijom izleganja larvi (ovicidnom aktivnošću), koja je varirala od 29,0 do 88,7% u zavisnosti od primenjene koncentracije (Tabela 2). Aktivnost je bila visoka (70%) i pri relativnoj niskoj koncentraciji od 0,049 mg/mL, što je rezultiralo i veoma niskoj dobijenoj IC₅₀ vrednosti od 0,04 mg/mL. Pri svim testiranim koncentracijama, efekat je bio značajno veći ($p < 0,05$) od obe negativne kontrole, dok je pri najvišoj koncentraciji od 50 mg/mL bio sličan pozitivnoj kontroli ($p > 0,05$). Antihelmintička aktivnost je bila u velikoj meri dozno-zavisna, s obzirom na vrednost R² od 0,90.

Tabela 2 Procentualna efikasnost (aritmetička sredina ± standardna devijacija) etarskog ulja korijandera protiv izleganja larvi gastrointestinalnih nematoda ovaca pri različitim koncentracijama

Koncentracija [mg/mL]	Inhibicija izleganja larvi (%)
50	88,7 ± 1,53 ^{AB}
12,5	85,7 ± 1,16 ^B
3,125	81,0 ± 1 ^{BC}
0,781	77,3 ± 2,08 ^{CD}
0,195	76,0 ± 4,36 ^{CD}
0,049	70,0 ± 1 ^D
0,025	36,3 ± 4,16 ^E
0,0125	29,0 ± 2 ^E
Tiabendazol, 0,025	96,3 ± 1,53 ^A
Tiabendazol, 0,0125	95,0 ± 1 ^A
3% Tween, v/v	14,2 ± 3,34 ^F
Destilovana H ₂ O	6,6 ± 1,92 ^F

*Vrednosti sa drugačijim označenim velikim slovima označavaju statistički značajnu razliku ($p < 0,05$)

DISKUSIJA

Usled razvoja i širenja antihelmintičke rezistencije i sve većih posledičnih ekonomskih gubitaka, raste interesovanje za alternativne strategije lečenja nematodnih infekcija kod životinja. U tom kontekstu mnogi istraživači se slažu da bioaktivni sastojci proizvedeni od lekovitog bilja predstavljaju obećavajuću alternativu konvencionalnim antihelmintičkim preparatima, ili važan dodatak u paleti preparata i metoda u integrisanom pristupu kontrole ovih parazita (Macedo i sar., 2010; Borges i Borges, 2016; Zeineldin i sar., 2018). Međutim, pre uvođenja pojedinih biljnih preparata za šire korišćenje u praksi, potrebno je dokazati

njihovu efikasnost uz pomoć pouzdanih testova. Pri tome su *in vitro* testovi veoma važni za inicijalnu procenu antihelmintičkog potencijala neke supstance i osnov za njihovu selekciju za dalja *in vivo* ispitivanja i studije toksičnosti (Fonseca i sar., 2013). Test izleganja larvi se smatra pouzdanim i preciznim testom koji je preporučen i naširoko se koristi kako za detekciju antihelmintičke rezistencije kod komercijalnih preparata, tako i za otkrivanje novih antihelmintičkih supstanci poput etarskih ulja (Coles i sar., 1992; André i sar., 2018; Štrbac i sar., 2022b).

Rezultati ovog istraživanja su dokazali antihelmintičku aktivnost etarskog ulja korijandera protiv gastrointestinalnih nematoda ovaca. Iako nije dostignuta maksimalna efikasnost pri ispitanim koncentracijama, ovoidna aktivnost je bila visoka pri čak šest testiranih koncentracija, odnosno od 0,049 do 50 mg/mL (70,0-88,7%). Zbog toga je IC_{50} vrednost (parametar koji se često koristi u ovakvim ispitivanjima kao pokazatelj efikasnosti i za poređenje sa drugim rezultatima), bila veoma niska (0,04 mg/mL) što ukazuje na veoma visoku ovoidnu aktivnost ulja korijandera. Ukoliko se ovaj rezultat uporedi sa drugim do sada testiranim uljima (André i sar., 2018; Štrbac i sar., 2022b), zapaža se da je to jedan od najefikasnijih, što govori u prilog visokom antihelmintičkom potencijalu korijandera i pogodnosti za dalja ispitivanja.

Antihelmintički efekat etarskog ulja korijandera protiv gastrointestinalnih nematoda ovaca je dokazan i u drugim istraživanjima. Dokazan je njegov ovoidni efekat protiv *Haemonchus contortus* od 14,8 - 99,0% pri koncentracijama od 0,15 - 2,5 mg/mL (IC_{50} 0,63 mg/mL) u istraživanju Macedo i sar. (2013). Iako nešto slabiji, u istom istraživanju je dokazan i larvicidni efekat ulja korijandera od 10,1 - 99,5% pri koncentracijama od 1,25-20,0 mg/mL (IC_{50} 2,89 mg/mL), sugerišući na njegovu aktivnost protiv različitih stadijuma parazita. Dokazana je i inhibitorna aktivnost korijandera na pokretljivost larvi različitih vrsta gastrointestinalnih nematoda, najjača protiv *Teladorsagia circumcincta*, *Trichostrongylus vitrinus*, *T. axei* i *H. contortus* sa IC_{50} vrednostima od 0,11, 0,15, 0,25 i 0,26% (u finalnoj koncentraciji), redom, dok je kod mešane infekcije IC_{50} vrednost bila 0,22% (Helal i sar., 2020). U našem istraživanju je takođe bila u pitanju mešana infekcija, a koprokultura je ispitana u prethodnom istraživanju gde su korišćene iste farme, sa sledećom ukupnom procentualnom zastupljenošću pojedinih rodova: *Haemonchus* 53%, *Trichostrongylus* 29,5%, *Teladorsagia* 14,5% i *Chabertia* 3% (Štrbac i sar., 2022a). Iz ovih rezultata se može zaključiti da korijander poseduje antihelmintičku efikasnost protiv različitih vrsta gastrointestinalnih nematoda.

Visoka antihelmintička aktivnost ulja korijandera potiče od njegovog hemijskog sastava. Ukoliko se posmatraju rezultati gasne hromatografije i masene spektrometrije, zapaža se prisustvo terpenskih i terpenoidnih jedinjenja iz

različitih hemijskih grupa. Ubedljivo najzastupljeniji sastojak je linalool (84,53%), aciklični monoterpenoidni alkohol, a pored njega i ugljovodonični terpeni (γ -terpinen, α -pinen, limonen i p-cimen), kao i terpenoidni keton kamfor. S obzirom da biološke osobine etarskih ulja u najvećoj meri potiču od njihovog glavnog sastojka (Dhifi i sar., 2016), može se konstatovati da efekat korijandera potiče od linaloola, ali je prisustvo drugih pomenutih jedinjenja bitno za ukupan sinergistički efekat. Visoka ovoidna aktivnost izolovanog linaloola protiv *H. contortus* je dokazana u istraživanju Katiki i sar. (2017) sa IC_{50} od 0,29 mg/mL, a linalool je pojedinačno gledano bio glavni sastojak *Arisaema franchetianum* (8,89%) i značajan sastojak *Arisaema lobatum* (6,67%), etarskih ulja sa ogromnim brojem izolovanih jedinjenja (preko 50), a koja su pokazala ovoidnu i larvicidnu aktivnost protiv *H. contortus* (Zhu i sar., 2013). Interesantno, sam izolovan linalool je u ovom istraživanju pokazao slabiju aktivnost u poređenju sa celim uljima, što ukazuje na značaj prisustva drugih jedinjenja i sinergističkog efekta.

Ukoliko se uporede rezultati analize hemijskog sastava etarskog ulja korijandera sa drugim istraživanjima, zapažaju se visoke podudarnosti. Tako su u istraživanju Helal i sar. (2020) glavni sastojci bili linalool (68,03%), kamfor (11,76%), γ -terpinen (10,48%) i α -pinen (9,71%), dok su u istraživanju Macedo i sar. (2013) bili β -linalool (73,21%), kamfor (4,25%), α -pinen (4,20%) i terpinen (3,10%). Ukoliko se pogledaju i druga istraživanja, hemijski sastav je takođe sličan uz dominaciju alkohola linalool, geraniol i terpinen-4-ol-a; ugljovodonika γ -terpinena, cimena, limonena i α -pinena; ketona kamfora, ali i estara geraniol acetata i linalil acetata (Mandal i Mandal, 2015). Iako je hemijski sastav suštinski sličan u različitim istraživanjima, ipak se zapažaju određene razlike, najčešće u procentualnoj zastupljenosti pojedinih jedinjenja. Ove razlike se mogu objasniti različitim faktorima poput mesta uzgoja biljke (padavine, svetlost, zemljište - pH, struktura, salinitet), dela biljke iz koga se ekstrahuje, starosti biljke i samog načina ekstrakcije (Fokou i sar., 2020), a treba imati u vidu da mogu dovesti i do određenih razlika u samoj biološkoj aktivnosti ulja.

Upotreba različitih biljaka i njihovih proizvoda poput etarskih ulja protiv gastrointestinalnih nematoda, ali i parazita generalno, ima brojne prednosti. Njihov bogat hemijski sastav sa jedinjenjima iz različitih hemijskih grupa i potencijalno različitim mehanizmom delovanja može doprineti visokoj antiparazitskoj aktivnosti, ali i manjoj podložnosti razvoju rezistencije. Sa druge strane, njihovo prirodno poreklo se povezuje sa nižim stepenom toksičnosti za domaćine, manjim količinama rezidua u mesu i mleku, kao i boljoj ekološkoj prihvatljivosti u poređenju sa hemijskim preparatima. Na kraju, veliki broj raspoloživih biljnih vrsta pogoduje širokoj upotrebi botaničkih antihelmintika, lakoj dostupnosti i pogodnoj ceni u državama sa razvijenim biodiverzitetom

(Ferreira i sar., 2018; Štrbac i sar., 2022b). Ipak, neophodna su dalja ispitivanja kako bi biljke i njihovi proizvodi našli široku primenu u veterinarskoj medicini.

ZAKLJUČAK

Razvoj i širenje antihelmintičke rezistencije i sve veći posledični ekonomski gubici zahtevaju potragu za alternativnim sredstvima, gde spadaju i različiti botanički antihelmintici. U ovom istraživanju, etarsko ulje korijandera je pokazalo visok antihelmintički potencijal s obzirom na ovoidni efekat utvrđen kod gastrointestinalnih nematoda ovaca, zbog čega se može smatrati pogodnim za dalja ispitivanja. Visoka ovoidna aktivnost korijandera potiče od bogatog hemijskog sastava, pre svega linaloolu uz još neka jedinjenja poput γ -terpinena, kamfora i α -pinena. Rezultati ove studije su još jedan pokazatelj moguće uloge fitoterapije u budućim održivim pristupima kontroli gastrointestinalnih nematoda ovaca, smanjenju upotrebe komercijalnih preparata i borbi protiv antihelmintičke rezistencije.

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LITERATURA

- André W. P. P., Ribeiro W. L. C., de Oliveira L. M. B., Macedo I. T. F., Rondon F. C. R., Bevilaqua C. M. L. (2018): Essential oils and their bioactive compounds in the control of gastrointestinal nematodes of small ruminants. *Acta Scientiae Veterinariae*, 46:1522.
- Belecké A., Kupčinskas T., Stadalienė I., Höglund J., Thamsborg S. M., Stuen, S., Petkevičius S. (2021): Anthelmintic resistance in small ruminants in the Nordic-Baltic region. *Acta Veterinaria Scandinavica*, 63:18.
- Borges D. G. L., Borges F. A. (2016): Plants and their medicinal potential for controlling gastrointestinal nematodes in ruminants. *Nematoda*, 3:e92016.
- Bosco A., Kießler J., Amadesi A., Varady M., Hinney B., Ianniello D., Paola Maurelli M., Cringoli G., Rinaldi L. (2020): The threat of reduced efficacy of anthelmintics against gastrointestinal nematodes in sheep from an area considered anthelmintic resistance-free. *Parasites & Vectors*, 13:457.
- Bosco A., Maurelli M. P., Ianniello D., Morgoglione M. E., Amadesi A., Coles G. C., Cringoli G., Rinaldi L. (2018): The recovery of added nematode eggs

- from horse and sheep faeces by three methods. *BMC Veterinary Research*, 14:7.
- Butnariu M., Sarac I. (2018): Essential oils from plants. *Journal of Biotechnology and Biomedical Science*, 1(4):35-43.
- Calvete C., González J. M., Ferrer L. M., Ramos J. J., Lacasta D., Delgado I., Uriarte J. (2020): Assessment of targeted selective treatment criteria to control subclinical gastrointestinal nematode infections on sheep farms. *Veterinary Parasitology*, 277:109018.
- Chahal K. K., Singh R., Kumar A., Bhardwaj U. (2017): Chemical composition and biological activity of *Coriandrum sativum L.*: A review. *Indian Journal of Natural Products and Resources*, 8(3):193-203.
- Coles G. C., Bauer C., Borgsteede F. H., Geerts S., Klei T. R., Taylor M. A., Waller P. J. (1992): World association for the advancement of veterinary parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary Parasitology*, 44(1-2):35-44.
- Dhifi W., Bellili S., Jazi S., Bahloul N., Mnif W. (2016): Essential oils' chemical characterization and investigation of some biological activities: a critical review. *Medicines (Basel)*, 3(4):25.
- Dyary H. O. (2018): Anthelmintic resistance of gastrointestinal nematodes insheep in Piramagroon sub-district, Sulaymaniyah/Iraq. *Tropical Biomedicine*, 35(2):373-382.
- Ferreira L. E., Benincasa B. I., Fachin A. L., Contini S. H. T., França S. C., Chagas A. C. S., Belebony R. O. (2018): Essential oils of *Citrus aurantifolia*, *Anthemis nobile* and *Lavandula officinalis*: In vitro anthelmintic activities against *Haemonchus contortus*. *Parasites & Vectors*, 11(1):269.
- Fokou J. B. H., Dongmo P. M. J., Boyom F. F. (2020): Essential oil's chemical composition and pharmacological properties. In *Essential oils - oils of nature*. Eds. H. El-Shemy, Intechopen, Chapter 2.
- Fonseca Z. A. A. S., Coelho W. A. C., Andre W. P. P., Ribeiro W. L. C., Bessa E. N., Galindo V. R., Pereira J. S., Ahid S. M. M. (2013): Use of herbal medicines in control of gastrointestinal nematodes of small ruminants: Efficacies and prospects. *Brazilian Journal of Hygiene and Animal Sanity*, 7(2):233-249.
- Giovanelli F., Mattellini M., Fichi G., Flamini G., Perrucci S. (2018). In vitro anthelmintic activity of four plant-derived compounds against sheep gastrointestinal nematodes. *Veterinary Sciences*, 5(3):78.
- Helal M. A., Abdel-Gawad A. M., Kandil O. M., Khalifa M. M. E., Cave G. W. V., Morrison A. A., Bartley D. J., Elsheikha H. M. (2020): Nematocidal
-

- effects of a coriander essential oil and five pure principles on the infective larvae of major ovine gastrointestinal nematodes in vitro. *Pathogens*, 9(9):740.
- Kaplan R. M. (2020): Biology, epidemiology, diagnosis, and management of anthelmintic resistance in gastrointestinal nematodes of livestock. *Veterinary Clinics of North America: Food Animal Practice*, 36(1):17-30.
- Katiki L. M., Barbieri A. M. E., Araujo R. C., Veríssimo C. J., Louvandini H., Ferreira J. F. S. (2017): Synergistic interaction of ten essential oils against *Haemonchus contortus* in vitro. *Veterinary Parasitology*, 243:47-51.
- Keeton S. T. N. (2016): Integrated methods for controlling gastrointestinal nematode infections in ewes and lambs. Louisiana State University and Agricultural and Mechanical College.
- Macedo I. T. F., Bevilaqua C. M. I., de Oliveira L. M. B., Camurça-Vasconcelos A. L. F., Vieira L. S., Oliveira F. R., Queiroz-Junior E. M., Tome A. R., Nascimento N. R. F. (2010): Anthelmintic effect of Eucalyptus staigeriana essential oil against goat gastrointestinal nematodes. *Veterinary Parasitology*, 173(1-2):93-98.
- Macedo I. T. F., de Oliveira L. M. B., Camurca-Vasconcelos A. L. F., Ribeiro W. L.C., dos Santos J. M. L., de Moraes S. M., de Paula H. C. B., Bevilaqua C. M. L. (2013): In vitro effects of *Coriandrum sativum*, *Tagetes minuta*, *Alpinia zerumbet* and *Lantana camara* essential oils on *Haemonchus contortus*. *Brazilian Journal of Veterinary Parasitology*, 22(4):463-469.
- Mandal S., Mandal M. (2015): Coriander (*Coriandrum sativum* L.) essential oil: Chemistry and biological activity. *Asian Pacific Journal of Tropical Biomedicine*, 5(6):421-428.
- Mucha W., Witkowska D. (2021): The applicability of essential oils in different stages of production of animal-based foods. *Molecules*, 26(13):3798.
- Pinto N. B., de Castro L. M., Azambuja R. H. M., Capella G. D. A., de Moura M. Q., Terto W. D., Freitag R. A., Jeske S. T., Villela M. M., Cleff M. B., Leite F. P. L. 2019. Ovicidal and larvicidal potential of *Rosmarinus officinalis* to control gastrointestinal nematodes of sheep. *Brazilian Journal of Veterinary Parasitology*, 28(4):807-811.
- Szewc M., De Waal T., Zintl A. (2021): Biological methods for the control of gastrointestinal nematodes. *The Veterinary Journal*, 268:105602.
- Štrbac F., Bosco A., Maurelli M. P., Ratajac R., Stojanović D., Simin N., Orčić D., Pušić I., Krnjajić S., Sotiraki S., Saralli S., Cringoli G., Rinaldi L. (2022a): Anthelmintic properties of essential oils to control gastrointestinal nematodes in sheep - in vitro and in vivo studies. *Veterinary Sciences*, 9(2):93.
-

- Štrbac F., Bosco A., Rinaldi L., Cringoli C., Stojanović D., Simin N., Orčić D., Pušić I., Ratajac R. (2022b): The use of essential oils against sheep gastrointestinal nematodes. In Animal health perspectives. Eds. R.Z. Abbas, A. Khan, P. Liu, M.K. Saleemi, Unique Scientific Publishers, Chapter 43.
- Štrbac F., Petrović K., Stojanović D., Ratajac R. (2021): Possibilities and limitations of the use of essential oils in dogs and cats. *Veterinary Journal of Republic of Srpska (Banja Luka)*, XXI(1-2):238-251.
- Zeineldin M., Abdelmegeid M., Barakat R., Ghanem M. (2018): A review: herbal medicine as an effective therapeutic approach for treating digestive disorders in small ruminants. *Alexandria Journal of Veterinary Sciences*, 56(1):33-44.
- Zhu L., Dai J., Yang L., Qui J. (2013): Anthelmintic activity of *Arisaema franchetianum* and *Arisaema lobatum* essential oils against *Haemonchus contortus*. *Journal of Ethnopharmacology*, 148(1):311-316.

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Original Scientific Paper**ANTIHELMINTIC POTENTIAL OF CORIANDER ESSENTIAL OIL
(*CORIANDRUM SATIVUM L.*) IN SHEEP****Filip ŠTRBAC^{1*}, Antonio BOSCO³, Kosta PETROVIĆ², Dragica STOJANOVIĆ², Radomir RATAJAC⁴, Nataša SIMIN⁵, Dejan ORČIĆ⁵, Giuseppe CRINGOLI³, Laura RINALDI³**

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Summary

The development of anthelmintic resistance in sheep gastrointestinal nematodes to commercial anthelmintics has led to enormous economic losses in many parts of the world. For this reason, new sustainable strategies for the control of these parasites are being actively searched, which include the use of different alternatives such as plant essential oils. The aim of this study was to determine the chemical composition of the essential oil of coriander (*Coriandrum sativum L.*) and to test its anthelmintic potential against gastrointestinal nematodes of sheep. Qualitative and semi-quantitative chemical characterization of the essential oil was performed using gas chromatography coupled with mass spectrometric detection (GC-MS analysis). The anthelmintic potential of the essential oil was tested using the *in vitro* egg hatch test (EHT), which was carried out at eight different concentrations of the tested oil (50; 12.5; 3.125; 0.781; 0.195; 0.049; 0.025 and 0.0125 mg/mL). Thiabendazole in concentrations of 0.025 and 0.0125 mg/mL was used as the positive control, and the emulsifier 3% Tween 80 v/v and distilled water was used as the negative control. GC-MS analyzes revealed a rich chemical composition of the tested oil, where a total of 17 different ingredients from different chemical groups were found, of which 15 were identified. The

dominant ingredient was linalool (84.5%) along with γ -terpinene (5.04%), camphor (3.26%) and α -pinene (2.88%). The EHT results showed a high anthelmintic potential of coriander oil with inhibition of egg hatching, i.e. an ovicidal effect of 29.0-88.7% depending on the concentration, with an IC_{50} concentration of 0.04 mg/mL and a clear dose-dependent effect ($R^2= 0.90$). This study confirmed the possible importance of botanical anthelmintics in the sustainable control of nematodes in animals and the combatting anthelmintic resistance. The obtained results indicate a possible role of coriander in future therapeutic approaches to infections caused by gastrointestinal nematodes of sheep, after further *in vivo* tests and toxicity studies.

Keywords: gastrointestinal nematodes, anthelmintic resistance, essential oil, coriander, gas chromatography, egg hatch test.

INTRODUCTION

Gastrointestinal nematodes lead to negative effects in sheep in various ways, from subclinical weight loss and decreased production of milk, meat and wool, to the appearance of anemia, diarrhea, anorexia, protein losses, reduced fertility, and in severe cases, even death (Giovanelli et al., 2018; Bosco et al., 2020; Beleckè et al., 2021). Control of infections caused by these parasites still mostly relies almost exclusively on the use of commercial anthelmintic drugs such as benzimidazoles, macrocyclic lactones and imidazothiazoles (Dyary, 2018). Although these drugs have been successfully used for decades in the therapy of the mentioned infections (Kaplan, 2020), their irrational use in terms of frequent and intensive treatments, high or low doses or consecutive use of anthelmintics from only one class led to the development of resistance in gastrointestinal nematodes (Bosco and et al., 2018; Pinto et al., 2019; Beleckè et al., 2021). The development of resistance has led to a decline in the effectiveness of the mentioned drugs and consequent large economic losses due to reduced production in animals and deaths, as well as treatment costs (Szewc et al., 2021). Due to all of the above, gastrointestinal nematodes represent one of the biggest obstacles facing modern sheep farming (Giovanelli et al., 2018).

The growing and more pronounced problem of resistance requires the design of new strategies for the control of the mentioned parasites, which today are mainly based on an integrated approach to therapy (Keeton, 2016). This approach implies, on the one hand, the rational use of anthelmintics, i.e. targeted treatments (treatment of only certain herds) and targeted selective treatments (treatment of only certain animals that require treatment within the herd), with a combination of several anthelmintics or their rotation (Calvete et al., 2020). On the other hand,

along with the rational use of commercial preparations, an integrated approach implies the introduction of various alternatives such as the selection of genetically resistant animals, pasture management, nutritional manipulation, biological control (use of nematophagous fungi or bacteria), development of vaccines as well as the use of botanical anthelmintics (Zeineldin et al., 2018; Pinto et al., 2019). At the same time, among the listed alternatives, phytotherapy is considered particularly promising in view of the obtained results (Borges and Borges, 2016). It implies the use of whole plants or their parts in animal feed, but also different plant products such as extracts and essential oils.

Essential oils represent aromatic, concentrated and complex mixtures of volatile and non-polar compounds isolated from plant material (Štrbac et al., 2022a). They are present in specialized cells or glands of certain plants and play a role in protecting them from predators and vermin, as well as in attracting pollinators (Butnariu and Sarac, 2018). In veterinary medicine, essential oils are increasingly used in the treatment and prevention of various diseases, although primarily in monogastric animals such as pigs and poultry (Mucha and Witkovska, 2021), but also in dogs and cats (Štrbac et al., 2021). However, there is more and more evidence about the anthelmintic effect of essential oils against gastrointestinal nematodes of sheep (Štrbac et al., 2022b). Their biological effect originates from their rich chemical composition and compounds belonging to different groups such as terpenes, terpenoids or phenylpropanoid compounds (Dhifi et al., 2016, Fokou et al., 2020).

Coriander (*Coriandrum sativum L.*) is an aromatic, herbaceous annual plant from the *Apiaceae* family (Chahal et al., 2017). It has a long history of use in cooking as a source of aromatic compounds with antibacterial, antifungal and antioxidant activity, so it is useful in food preparation as an aroma and auxiliary agent (Mandal and Mandal, 2015). It is also known for its wide range of medicinal properties and is used for various gastrointestinal disorders such as anorexia, dyspepsia, flatulence, diarrhea and vomiting. Coriander fruit is also known as a cooling agent, tonic, diuretic and aphrodisiac, while its essential oil is considered useful in flatulence, rheumatism and neuralgia, with known antimicrobial, antioxidant and pesticidal properties (Chahal et al., 2017).

The aim of this study was to examine the anthelmintic activity of coriander essential oil against gastrointestinal nematodes of sheep, with the determination of its chemical composition and the identification of compounds significant for anthelmintic activity.

MATERIALS AND METHODS

Analysis of chemical composition

Coriander essential oil was purchased from the Institute of Field and Vegetable Crops in Novi Sad. Qualitative and semi-quantitative chemical characterization of the essential oil was performed at the Faculty of Science in Novi Sad using gas chromatography coupled with mass spectrometric detection (GC-MS). In the gas chromatograph (Agilent Technologies series 6890), 1 μ L of the solution of the tested oil (10 μ L/mL in hexane) was injected in split mode, with a split ratio of 1:10, at a temperature of 250°C. The components were separated on a non-polar poly (tetramethyl-1,4-silphenylenesiloxane) column HP-5 ms (Agilent Technologies) with dimensions 30m \times 0.25mm, layer thickness 0.25 μ m. The column was eluted in a temperature-programmed regime, with a starting temperature of 50°C, ramp 8°C/min to 120°C, 15°C/min to 230°C, 20°C/min to 270°C/min, and retention at the final temperature of 16.92 min (total analysis time 35 min). High-purity helium (5.0) was used as carrier gas in a constant flow regime of 1.0 mL/min. The effluent was sent via a transfer line maintained at 280°C to a mass spectrometer with electron ionization (Agilent Technologies series 5975). The mass spectrometer parameters were: electron energy 70eV, ion source temperature 230°C, quadrupole temperature 150°C. Scan acquisition mode was applied, in the m/z range 35-400, and with a solvent delay of 2.30 min. In order to achieve a better agreement between the experimental and library spectra, the standard spectra tune was used. Data were processed using Agilent Technologies MSD ChemStation software (revision E01.01.335) in combination with AMDIS (ver. 2.64) and NIST MS Search software (ver. 2.0d). AMDIS was used for deconvolution of mass spectra of coeluting compounds, and NIST MS Search provided a library search algorithm complementary to ChemStation's PBM algorithm. Wiley Registry of Mass Spectral Data 7th Edition and NIST/EPA/NIH Mass spectral Spectral Library 05 libraries were used to identify mass spectra. The identity of the compound was confirmed by comparing the linear retention indices with literature data. The relative proportions of the compounds were determined by the normalization method, based on the area of the peaks in TIC.

Egg hatch test

The anthelmintic potential of coriander essential oil against gastrointestinal nematodes of sheep was tested using the egg hatch test (EHT), which measures the effect of the application of a substance on the inhibition of hatching of larvae from parasite eggs, i.e. the ovicidal effect. The study was conducted in the regional center for monitoring parasitosis KREMOPAR (Eboli, Salerno, southern

Italy). For the isolation of nematode eggs, samples of sheep feces taken directly from the rectal ampoule, from sheep from two separate farms in this region, were used, and the nematode eggs were isolated using the so-called recovery method (Bosco et al., 2018).

Initially, feces samples were homogenized and filtered under flowing water through sieves with openings of different sizes (1 mm, 250 μ m, 212 μ m and 38 μ m), in order to separate eggs from feces. The eggs retained on the sieve of the smallest size were then washed with distilled water into test tubes and centrifuged at 1500 RPM, after which the precipitate was removed. After that, centrifugation was performed so that the eggs that floated to the surface were isolated in new test tubes to which distilled water was added. Finally, the obtained solutions were centrifuged several more times to remove debris and obtain an aqueous solution with eggs.

Egg hatch test was conducted according to the instructions from the literature (Ferreira et al., 2018) with certain modifications. Eight different concentrations (50; 12.5; 3.125; 0.781; 0.195; 0.049; 0.025 and 0.0125 mg/mL) of coriander oil were tested using well plates. In the wells, solutions of the mentioned decreasing concentrations of oils emulsified in 3% Tween 80, v/v, were made, and then supplemented with the obtained aqueous solution with eggs (about 40 μ l, i.e. 100-150 eggs per well) and distilled water to a total amount of 0.5 mL per well.

The required amount of oil for the highest concentration was determined by calculation, and then the other concentrations were obtained using the dilution method.

Thiabendazole in concentrations of 0.025 mg/mL and 0.0125 mg/mL was used as a positive control, and 3% Tween 80, v/v and distilled water were used as negative controls. All oil concentrations and controls were tested in a total of three replicates. After making the mentioned solutions, the plate with wells was incubated at 27°C for 48 hours, and after that 1-2 drops of Lugol's solution were added to each well, in order to stop the hatching of nematode larvae from the eggs. The prepared solutions were then observed under a microscope with the aim of counting eggs and hatched larvae at each concentration of oil and controls.

Statistical processing

The percentage of inhibition of egg hatching into the larval form was calculated using the following formula (Coles et al., 1992; Pinto et al., 2019):

$$IH = \text{number of eggs} / (\text{number of eggs} + \text{number of larvae}) \times 100\%$$

After that, the final value for each concentration and control was calculated as the arithmetic mean between three repetitions. One-factor analysis of variance with post hoc Tukey test ($p < 0.05$) was used to compare the obtained values between

different concentrations of the tested oil, as well as to compare them with the controls. On the other hand, nonlinear regression and logarithmic distribution were used to determine the concentration that inhibits 50% of eggs from developing into larval form (Ferreira et al., 2018; Štrbac et al., 2022a). Statistical analyzes were performed in the GraphPad Prism 8.4.3 program (GraphPad Holdings, LLC, San Antonio, CA, USA).

RESULTS

Analysis of chemical composition

The results of the chemical analysis of the composition obtained by gas chromatography and mass spectrometry showed a rich chemical composition of coriander essential oil. The total number of compounds was 17, of which 15 were identified (Table 1), from different chemical groups, and the dominant compounds were linalool (84.53%), γ -terpinene (5.08%), camphor (3.26%)) and α -pinene (2.88%).

Table 1. Chemical composition of coriander essential oil determined by gas chromatography and mass spectrometry

Arithmetic retention index	Compound	% Total peak area
925	α -Thujone	0.02
932	α -Pinene	2.88
946	Camphene	0.30
971	Sabinene	0.02
976	β -Pinene	0.21
989	Myrcene	0.37
1016	α -Terpinene	0.05
1023	p-Cymene	1.08
1027	Limonene	1.12
1029	1,8-Cineol	0.14
1056	γ -Terpinene	5.08
1087	Terpinolene	0.30
1100	Linalool	84.53
1143	Camphor	3.26
1165	<i>Unknown</i>	0.12
1176	Terpinen-4-ol	0.27
1191	<i>Unknown</i>	0.16

Egg hatch test

Coriander essential oil showed high anthelmintic potential against gastrointestinal nematodes of sheep with inhibition of larval hatching (ovicidal activity), which varied from 29.0 to 88.7% depending on the applied concentration (Table 2). The activity was high (70%) even at a relatively low concentration of 0.049 mg/mL, which resulted in a very low obtained IC₅₀ value of 0.04 mg/mL. At all tested concentrations, the effect was significantly greater ($p < 0.05$) than both negative controls, while at the highest concentration of 50 mg/mL it was similar to the positive control ($p > 0.05$). Anthelmintic activity was largely dose-dependent, with an R² value of 0.90.

Table 2. Percentage efficacy (arithmetic mean \pm standard deviation) of coriander essential oil against the hatching of sheep gastrointestinal nematode eggs at different concentrations

Concentration [mg/mL]	Inhibition of hatchability (%)
50	88.7 \pm 1.53 ^{AB}
12.5	85.7 \pm 1.16 ^B
3.125	81.0 \pm 1 ^{BC}
0.781	77.3 \pm 2.08 ^{CD}
0.195	76.0 \pm 4.36 ^{CD}
0.049	70.0 \pm 1 ^D
0.025	36.3 \pm 4.16 ^E
0.0125	29.0 \pm 2 ^E
Thiabendazole, 0.025	96.3 \pm 1.53 ^A
Thiabendazole, 0.0125	95.0 \pm 1 ^A
3% Tween, v/v	14.2 \pm 3.34 ^F
Distilled H ₂ O	6.6 \pm 1.92 ^F

* Values with different capital letters indicate a statistically significant difference ($p < 0.05$)

DISCUSSION

Due to the development and spread of anthelmintic resistance and the increasing consequent economic losses, there is a growing interest in alternative strategies for the treatment of nematode infections in animals. In this context, many researchers agree that bioactive ingredients produced from medicinal plants represent a promising alternative to conventional anthelmintic preparations, or an important addition to the range of preparations and methods in an integrated approach to the control of these parasites (Macedo et al., 2010; Borges and Borges, 2016; Zeineldin et al., 2018). However, before the introduction of individual herbal preparations for wider use in practice, it is necessary to prove their effectiveness with the assistance of reliable tests. At the same time, *in vitro*

tests are very important for the initial assessment of the anthelmintic potential of a substance and the basis for their selection for further *in vivo* tests and toxicity studies (Fonseca et al., 2013). The egg hatch test is considered a reliable and precise test that is recommended and widely used both for the detection of anthelmintic resistance in commercial preparations and for the discovery of new anthelmintic substances such as essential oils (Coles et al., 1992; André et al., 2018; Štrbac et al., 2022b).

The results of this study proved the anthelmintic activity of coriander essential oil against gastrointestinal nematodes of sheep. Although maximum efficiency was not reached at the tested concentrations, ovicidal activity was high at even six tested concentrations, i.e. from 0.049 to 50 mg/mL (70.0-88.7%). Due to that, the IC₅₀ value (a parameter that is often used in such tests as an indicator of efficiency and for comparison with other results) was very low (0.04 mg/mL), which indicates a very high ovicidal activity of coriander oil. If this result is compared with other oils tested so far (André et al., 2018; Štrbac et al., 2022b), it can be observed that it is one of the most effective, which indicate the high anthelmintic potential of coriander and its convenience for further tests.

The anthelmintic effect of coriander essential oil against gastrointestinal nematodes of sheep has been proven in other studies. Its ovicidal effect against *Haemonchus contortus* of 14.8 - 99.0% at concentrations of 0.15 - 2.5 mg/mL (IC₅₀ 0.63 mg/mL) was proven in a study by Macedo et al. (2013). Although not so strong, in the same study, the larvicidal effect of coriander oil was also proven to be 10.1 - 99.5% at concentrations of 1.25-20.0 mg/mL (IC₅₀ 2.89 mg/mL), suggesting its activity against different stages of parasites. The inhibitory activity of coriander on the motility of larvae of different types of gastrointestinal nematodes was also proven, the strongest against *Teladorsagia circumcincta*, *Trichostrongylus vitrinus*, *T. axei* and *H. contortus* with IC₅₀ values of 0.11, 0.15, 0.25 and 0.26% (in the final concentration), respectively, while in mixed infection the IC₅₀ value was 0.22% (Helal et al., 2020). In our study, we also used mixed infection, and coproculture was examined in a previous study where the same farms were used, with the following percentage of individual genera: *Haemonchus* 53%, *Trichostrongylus* 29.5%, *Teladorsagia* 14.5% and *Chabertia* 3 % (Štrbac et al., 2022a). From these results, it can be concluded that coriander has anthelmintic efficacy against various types of gastrointestinal nematodes.

If the results of gas chromatography and mass spectrometry are observed, the presence of terpene and terpenoid compounds from different chemical groups be noticed. By far the most abundant ingredient is linalool (84.53%), an acyclic monoterpenoid alcohol, and next to it, hydrocarbon terpenes (γ -terpinene, α -pinene, limonene and p-cymene), as well as the terpenoid ketone camphor. Given

that the biological properties of essential oils derive to the greatest extent from their main ingredient (Dhifi et al., 2016), it can be concluded that the effect of coriander originates from linalool, but the presence of other mentioned compounds is essential for the overall synergistic effect. High ovicidal activity of isolated linalool against *H. contortus* was demonstrated in the study done by Katiki et al. (2017) with an IC_{50} of 0.29 mg/mL, and linalool individually was the main constituent of *Arisaema franchetianum* (8.89%) and a significant constituent of *Arisaema lobatum* (6.67%), essential oils with a huge number of isolated compounds (over 50), which showed ovicidal and larvicidal activity against *H. contortus* (Zhu et al., 2013). Interestingly, isolated linalool alone showed a weaker activity in this study compared to whole oils, which indicates the importance of the presence of other compounds and the synergistic effect.

If the results of the analysis of the chemical composition of coriander essential oil are compared with other researches, a high level of agreement is observed. Thus, in the research done by Helal et al. (2020) the main ingredients were linalool (68.03%), camphor (11.76%), γ -terpinene (10.48%) and α -pinene (9.71%), while in the research done by Macedo et al. (2013) the main ingredients were β -linalool (73.21%), camphor (4.25%), α -pinene (4.20%) and terpinene (3.10%). In other studies, the chemical composition is also similar with the dominance of alcohol linalool, geraniol and terpinen-4-ol; hydrocarbons γ -terpinene, cymene, limonene and α -pinene; camphor ketone, but also esters of geraniol acetate and linalyl acetate (Mandal and Mandal, 2015). Although the chemical composition is similar in different studies, certain differences are still observed, most often in the percentage of individual compounds. These differences can be explained by various factors such as the place where the plant is grown (rainfall, light, soil - pH, structure, salinity), the part of the plant from which it is extracted, the age of the plant and the method of extraction (Fokou et al., 2020). Those factors can lead to certain differences in the biological activity of the oil itself.

The use of different plants and their products, such as essential oils, against gastrointestinal nematodes, but also parasites in general, has numerous advantages. Their rich chemical composition with compounds from different chemical groups and potentially different mechanisms of action can contribute to a high antiparasitic activity, but also to a lower susceptibility to the development of resistance. On the other hand, their natural origin is associated with a lower degree of toxicity for hosts, smaller amounts of residues in meat and milk, as well as better environmental acceptability compared to chemical preparations. Finally, the large number of available plant species favors the wide use of botanical anthelmintics, easy availability and favorable price in countries with developed biodiversity (Ferreira et al., 2018; Štrbac et al., 2022b). However, further tests are

necessary in order for plants and their products to be widely used in veterinary medicine.

CONCLUSION

The development and spread of anthelmintic resistance and the increasing consequent economic losses require the search for alternative tools, which include various botanical anthelmintics. In this study, coriander essential oil showed a high anthelmintic potential with regard to the ovicidal effect found in sheep gastrointestinal nematodes, which is why it can be considered suitable for further tests. Coriander's high ovicidal activity originates from its rich chemical composition, primarily linalool along with other compounds such as γ -terpinene, camphor and α -pinene. The results of this study indicate the possible role of phytotherapy in future sustainable approaches to the control of gastrointestinal nematodes in sheep, reducing the use of commercial preparations and anthelmintic resistance.

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REFERENCES

- André W. P. P., Ribeiro W. L. C., de Oliveira L. M. B., Macedo I. T. F., Rondon F. C. R., Bevilaqua C. M. L. (2018): Essential oils and their bioactive compounds in the control of gastrointestinal nematodes of small ruminants. *Acta Scientiae Veterinariae*, 46:1522.
- Beleckè A., Kupčinskis T., Stadalienè I., Höglund J., Thamsborg S. M., Stuen, S., Petkevičius S. (2021): Anthelmintic resistance in small ruminants in the Nordic-Baltic region. *Acta Veterinaria Scandinavica*, 63:18.
- Borges D. G. L., Borges F. A. (2016): Plants and their medicinal potential for controlling gastrointestinal nematodes in ruminants. *Nematoda*, 3:e92016.
- Bosco A., Kießler J., Amadesi A., Varady M., Hinney B., Ianniello D., Paola Maurelli M., Cringoli G., Rinaldi L. (2020): The threat of reduced efficacy of anthelmintics against gastrointestinal nematodes in sheep from an area considered anthelmintic resistance-free. *Parasites & Vectors*, 13:457.
- Bosco A., Maurelli M. P., Ianniello D., Morgoglione M. E., Amadesi A., Coles G. C., Cringoli G., Rinaldi L. (2018): The recovery of added nematode eggs
-

- from horse and sheep faeces by three methods. *BMC Veterinary Research*, 14:7.
- Butnariu M., Sarac I. (2018): Essential oils from plants. *Journal of Biotechnology and Biomedical Science*, 1(4):35-43.
- Calvete C., González J. M., Ferrer L. M., Ramos J. J., Lacasta D., Delgado I., Uriarte J. (2020): Assessment of targeted selective treatment criteria to control subclinical gastrointestinal nematode infections on sheep farms. *Veterinary Parasitology*, 277:109018.
- Chahal K. K., Singh R., Kumar A., Bhardwaj U. (2017): Chemical composition and biological activity of *Coriandrum sativum L.*: A review. *Indian Journal of Natural Products and Resources*, 8(3):193-203.
- Coles G. C., Bauer C., Borgsteede F. H., Geerts S., Klei T. R., Taylor M. A., Waller P. J. (1992): World association for the advancement of veterinary parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Veterinary Parasitology*, 44(1-2):35-44.
- Dhifi W., Bellili S., Jazi S., Bahloul N., Mnif W. (2016): Essential oils' chemical characterization and investigation of some biological activities: a critical review. *Medicines (Basel)*, 3(4):25.
- Dyary H. O. (2018): Anthelmintic resistance of gastrointestinal nematodes insheep in Piramagroon sub-district, Sulaymaniyah/Iraq. *Tropical Biomedicine*, 35(2):373-382.
- Ferreira L. E., Benincasa B. I., Fachin A. L., Contini S. H. T., França S. C., Chagas A. C. S., Belebony R. O. (2018): Essential oils of *Citrus aurantifolia*, *Anthemis nobile* and *Lavandula officinalis*: In vitro anthelmintic activities against *Haemonchus contortus*. *Parasites & Vectors*, 11(1):269.
- Fokou J. B. H., Dongmo P. M. J., Boyom F. F. (2020): Essential oil's chemical composition and pharmacological properties. In *Essential oils - oils of nature*. Eds. H. El-Shemy, Intechopen, Chapter 2.
- Fonseca Z. A. A. S., Coelho W. A. C., Andre W. P. P., Ribeiro W. L. C., Bessa E. N., Galindo V. R., Pereira J. S., Ahid S. M. M. (2013): Use of herbal medicines in control of gastrointestinal nematodes of small ruminants: Efficacies and prospects. *Brazilian Journal of Hygiene and Animal Sanity*, 7(2):233-249.
- Giovanelli F., Mattellini M., Fichi G., Flamini G., Perrucci S. (2018). In vitro anthelmintic activity of four plant-derived compounds against sheep gastrointestinal nematodes. *Veterinary Sciences*, 5(3):78.
- Helal M. A., Abdel-Gawad A. M., Kandil O. M., Khalifa M. M. E., Cave G. W. V., Morrison A. A., Bartley D. J., Elsheikha H. M. (2020): Nematocidal
-

- effects of a coriander essential oil and five pure principles on the infective larvae of major ovine gastrointestinal nematodes in vitro. *Pathogens*, 9(9):740.
- Kaplan R. M. (2020): Biology, epidemiology, diagnosis, and management of anthelmintic resistance in gastrointestinal nematodes of livestock. *Veterinary Clinics of North America: Food Animal Practice*, 36(1):17-30.
- Katiki L. M., Barbieri A. M. E., Araujo R. C., Veríssimo C. J., Louvandini H., Ferreira J. F. S. (2017): Synergistic interaction of ten essential oils against *Haemonchus contortus* in vitro. *Veterinary Parasitology*, 243:47-51.
- Keeton S. T. N. (2016): Integrated methods for controlling gastrointestinal nematode infections in ewes and lambs. Louisiana State University and Agricultural and Mechanical College.
- Macedo I. T. F., Bevilaqua C. M. I., de Oliveira L. M. B., Camurça-Vasconcelos A. L. F., Vieira L. S., Oliveira F. R., Queiroz-Junior E. M., Tome A. R., Nascimento N. R. F. (2010): Anthelmintic effect of *Eucalyptus staigeriana* essential oil against goat gastrointestinal nematodes. *Veterinary Parasitology*, 173(1-2):93-98.
- Macedo I. T. F., de Oliveira L. M. B., Camurca-Vasconcelos A. L. F., Ribeiro W. L.C., dos Santos J. M. L., de Moraes S. M., de Paula H. C. B., Bevilaqua C. M. L. (2013): In vitro effects of *Coriandrum sativum*, *Tagetes minuta*, *Alpinia zerumbet* and *Lantana camara* essential oils on *Haemonchus contortus*. *Brazilian Journal of Veterinary Parasitology*, 22(4):463-469.
- Mandal S., Mandal M. (2015): Coriander (*Coriandrum sativum L.*) essential oil: Chemistry and biological activity. *Asian Pacific Journal of Tropical Biomedicine*, 5(6):421-428.
- Mucha W., Witkowska D. (2021): The applicability of essential oils in different stages of production of animal-based foods. *Molecules*, 26(13):3798.
- Pinto N. B., de Castro L. M., Azambuja R. H. M., Capella G. D. A., de Moura M. Q., Terto W. D., Freitag R. A., Jeske S. T., Villela M. M., Cleff M. B., Leite F. P. L. 2019. Ovicidal and larvicidal potential of *Rosmarinus officinalis* to control gastrointestinal nematodes of sheep. *Brazilian Journal of Veterinary Parasitology*, 28(4):807-811.
- Szewc M., De Waal T., Zintl A. (2021): Biological methods for the control of gastrointestinal nematodes. *The Veterinary Journal*, 268:105602.
- Štrbac F., Bosco A., Maurelli M. P., Ratajac R., Stojanović D., Simin N., Orčić D., Pušić I., Krnjajić S., Sotiraki S., Saralli S., Cringoli G., Rinaldi L. (2022a): Anthelmintic properties of essential oils to control gastrointestinal nematodes in sheep - in vitro and in vivo studies. *Veterinary Sciences*, 9(2):93.
-

- Štrbac F., Bosco A., Rinaldi L., Cringoli C., Stojanović D., Simin N., Orčić D., Pušić I., Ratajac R. (2022b): The use of essential oils against sheep gastrointestinal nematodes. In Animal health perspectives. Eds. R.Z. Abbas, A. Khan, P. Liu, M.K. Saleemi, Unique Scientific Publishers, Chapter 43.
- Štrbac F., Petrović K., Stojanović D., Ratajac R. (2021): Possibilities and limitations of the use of essential oils in dogs and cats. *Veterinary Journal of Republic of Srpska (Banja Luka)*, XXI(1-2):238-251.
- Zeineldin M., Abdelmegeid M., Barakat R., Ghanem M. (2018): A review: herbal medicine as an effective therapeutic approach for treating digestive disorders in small ruminants. *Alexandria Journal of Veterinary Sciences*, 56(1):33-44.
- Zhu L., Dai J., Yang L., Qui J. (2013): Anthelmintic activity of *Arisaema franchetianum* and *Arisaema lobatum* essential oils against *Haemonchus contortus*. *Journal of Ethnopharmacology*, 148(1):311-316.

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